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**REMARKS****Introduction**

Receipt is acknowledged of a non-final office action dated November 19, 2002. In the action, the Examiner rejected claims 1-3, 20-22, 24-26 and 28-30 for allegedly being drawn to non-statutory subject matter. The Examiner also rejected claims 1-4 and 20-42 for alleged nonenablement. Lastly, the Examiner rejected claims 1-4 and 20-43 as allegedly indefinite.

**Status of the Claims**

In this amendment, applicant amended claims 1-4, 20-23, 26, 30, 40, and 41, added new claims 44-49, and cancelled claims 42-43. Support for the claim amendments can be found throughout the specification, and in particular, on page 4, lines 4-25 (claim 3), page 6, line 1 (claims 1-4), page 6, lines 22-30, page 21, lines 3-6, and page 22, lines 8-10 (claims 1-4 and 20-23). Claims 26 and 30 are amended to correct typographical errors. Support for new claims 44-49 can be found in example 2 (claim 46), on page 3, lines 13-16 and 27-30 (claims 44-45 and 49), page 2, lines 28-29 (claim 47), page 3, lines 30-31 (claim 48) of the instant specification. Upon entry of this amendment, 1-4, 20-41 and 44-49 will be pending.

**35 U.S.C. § 101**

The Examiner rejected claims 1-3, 20-22, 24-26 and 28-30 for allegedly being drawn to non-statutory subject matter.

Applicants amended independent claims 1-4 to recite the term "isolated" in order to distinguish the claimed subject matter over the product of nature. Support for this amendment can be found on page 6, line 1.

**35 U.S.C. § 112, 1<sup>st</sup> paragraph**

The Examiner rejected claims 4, 23, 27, 31, 35 and 39 as allegedly non-enabled. In particular, the Examiner asserted that "there is no indication in the specification as to public availability [of the deposited FERM BP-6479 organism]." Office action at 5.

Submitted herewith is a statement by applicants' attorney which states that the FERM BP-6479 has been deposited under the Budapest Treaty and that all restrictions upon

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availability to the strain will be removed upon the granting of a patent from the above-identified application

Additionally, the Examiner rejected claims 1-3, 20-22, 24-26, 28-30, 32-34, 36-38 and 40-42 as allegedly non-enabled, stating that the specification "does not reasonably provide enablement for any DNA which encodes a mutated protein derived from SEQ ID NO: 2, or DNA which encodes a protein which has a certain degree of sequence similarity to SEQ ID NO: 2 or DNA which hybridizes to DNA encoding SEQ ID NO: 2 and encodes a protein that upon expression confers lysozyme insensitivity to strains that are lysozyme sensitive" (office action at 6). Essentially, the Examiner finds that "a single fully disclosed sequence does not support claims to DNA encoding any protein with a certain degree of sequence identity, given the lack of guidance regarding what sequences define the lysozyme insensitivity properties of SEQ ID NO: 2" (office action at 8).

Applicants respectfully disagree. It will be readily understood by a skilled artisan that numerous different nucleotide sequences could encode the same protein of the present invention as a result of the degeneracy of the genetic code. In addition, it will be understood that a skilled person may, using routine techniques, make nucleotide substitutions that do not affect the protein activity encoded by the nucleotide sequence of the present invention.

Thus, to limit the claims to a nucleotide sequence which is 100% identical to SEQ ID NO: 1, or a nucleotide sequence that encodes a protein that is 100% identical to SEQ ID NO: 2, would be unduly restrictive. A skilled person would readily be able to obtain sequences which are not identical to SEQ ID NO: 1 or SEQ ID NO: 2 but which would otherwise be suitable for carrying out the invention, without undue experimental burden. Thus, a skilled artisan could readily carry out the invention across the entire scope of the claims.

Nevertheless, applicants would like to draw the Examiner's attention to the specification beginning on page 3, line 34 to page 16, line 11. The specification discloses, in detail, how to make and use a DNA sequence that encodes a protein conferring lysozyme insensitivity on a lysozyme sensitive organism belonging to *Corynebacterium glutamicum*.

In addition, applicants would like to point the Examiner to Example 2 on page 21 of the present application. This example clearly demonstrates that a DNA sequence which hybridizes under stringent conditions to the DNA sequence identified as SEQ ID NO: 1, or

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encodes a protein that is 60% or more homologous to the amino acid sequence identified in SEQ ID NO: 2 is suitable for use in the present invention. In particular, Example 2, provides that "in the ORF [open reading frame] of 1920 bp, there is a mutation causative of lysozyme sensitivity of *Corynebacterium* KY9714 strain introduced in a SacI-KpnI fragment of about 1.2 kb" (specification at 23, lines 9-13). Indeed, when the ability of a deletion mutant to recover lysozyme sensitivity was examined, the results indicated that a DNA sequence represented as nucleotides 271 to 1593 in SEQ ID NO: 1 recovered lysozyme sensitivity of *Corynebacterium glutamicum*.

Accordingly, the Examiner is incorrect in asserting that the specification does not enable claims to DNA sequences, other than the DNA sequence provided in SEQ ID NO: 1, that confer lysozyme insensitivity. The DNA sequence that corresponds to positions 271 to 1593 encodes a 441 amino acid sequence, whereas SEQ ID NO: 1 encodes a 640 residue polypeptide (SEQ ID NO: 2). Therefore, the "271 to 1593 sequence" is not 100% identical to SEQ ID NO: 1, yet is still suitable for use in the present invention. In fact, because the "271 to 1593 sequence" and SEQ ID NO: 1 share a common sequence, the former can hybridize to SEQ ID NO: 1 under the hybridization conditions outlined in the instant application. See, specification at page 4, lines 11-17. Thus, the specification clearly teaches one of skill in the art how to make and use a DNA sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 and has the ability to recover lysozyme sensitivity.

Likewise, the 441 amino acid sequence encoded by the "271 to 1593 sequence" is not identical to the 640 amino acid sequence of SEQ ID NO: 2. Thus, the 441 amino acid sequence is homologous to, but not 100% identical to SEQ ID NO: 2. Specifically, the 441 amino acid sequence has 69% identity to the amino acid sequence of SEQ ID NO: 2 (441 amino acids/640 amino acids x 100). As such, the specification also discloses how to make a DNA sequence that encodes a protein that is at least 60% homologous to SEQ ID NO: 2 and use such a sequence to recover lysozyme sensitivity.

In other words, Example 2 shows that (i) a DNA sequence that comprises the nucleotide sequence of SEQ ID NO: 1 and encodes a protein having the amino acid sequence in SEQ ID NO: 2 can recover lysozyme sensitivity and (ii) a DNA sequence that encodes one

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or more amino acid deletion mutants of the amino acid sequence of SEQ ID NO: 2 can recover lysozyme sensitivity as well.

With regard to claim 3 and claims dependent therefrom, the Examiner points out that a DNA strand cannot both hybridize to the coding strand of protein XYZ and encode protein XYZ unless the DNA is palindromic (office action at 6-7). However, applicants' amendment to claim 3 obviates this rejection. Support for the amendment is found at page 4, lines 4-25 of the instant specification.

**35 U.S.C. § 112, 2<sup>nd</sup> paragraph**

The Examiner rejected claims 1-4 and 20-43 as allegedly indefinite. In particular, the Examiner indicated that "stringent hybridization conditions" and "sensitivity" or "insensitivity" to lysozyme is unclear (office action at 9). In addition, the Examiner asserted that it "is not stated and thus not clear...that the resistance/sensitivity is measured in cells cultured under the same conditions, as it is known in the art that culture conditions affect the sensitivity of *Corynebacterium* to lysozyme" (office action at 9).

In the interest of expediting prosecution, applicants cancelled claims 42-43, rendering the rejection of these claims for alleged indefiniteness moot.

With regard to "stringent hybridization conditions," the Examiner stated that while the stringent condition on page 4, lines 11-17 of the specification is noted, the stringent condition described at page 4, lines 11-17 is non-limiting (office action at 8). Applicants respectfully assert that the temperature and salt concentration necessary for hybridization are specifically defined and therefore, are not non-limiting. Nevertheless, applicants amended claim 3 to recite hybridization conditions that can be used to select a DNA that will hybridize to SEQ ID NO: 1. Therefore, the amendment to claim 3 obviates the instant rejection.

Concerning the phrase "insensitivity" to lysozyme as recited in the claims, applicants amended claims 1-4 to recite "an ability to grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine, and 100 µg/ml lysozyme" based on the description at page 6, lines 22-30, page 21, lines 3-6, and page 22, lines 8-10.

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Regarding the term "sensitivity," claims 20-23 have been amended to recite a lysozyme concentration of "50 µg/ml." In addition, claims 20-23 have been amended to recite "cannot grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine, and 50 µg/ml lysozyme".

Applicants trust that the amendments to claims 1-4 and 20-23 address the Examiner's concerns and the rejection should be withdrawn.

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**CONCLUSION**

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and arguments.

It is respectfully urged that the present application is now in condition for allowance. Early notice to that effect is earnestly solicited.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date May 19, 2003By Stephen B. Maebius

FOLEY & LARDNER  
Washington Harbour  
3000 K Street, N.W., Suite 500  
Washington, D.C. 20007-5143  
Telephone: (202) 672-5569  
Facsimile: (202) 672-5399

Stephen B. Maebius  
Attorney for Applicant  
Registration No. 35,264

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**MARKED UP VERSION SHOWING CHANGES MADE TO CLAIMS**

1. **(Twice Amended) An isolated [A] DNA encoding (a) a protein which comprises the amino acid sequence of SEQ ID NO: 2, or (b) a protein which comprises an amino acid sequence, wherein one or more amino acids are deleted, substituted, or added in the amino acid sequence of SEQ ID NO: 2, and confers an ability to grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine and 100 µg/ml lysozyme to a microorganism belonging to *Corynebacterium glutamicum* [and which has an activity of giving a lysozyme insensitivity to a lysozyme-sensitive microorganism belonging to *Corynebacterium glutamicum*].**
2. **(Amended) An isolated DNA encoding [coding for] a protein which comprises an amino acid sequence having 60% or more homology to the amino acid sequence of SEQ ID NO: 2 and confers an ability to grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine and 100 µg/ml lysozyme to a microorganism belonging to *Corynebacterium glutamicum* [which has an activity of giving a lysozyme insensitivity to a lysozyme-sensitive microorganism belonging to *Corynebacterium glutamicum*].**
3. **(Amended) An isolated [A] DNA comprising the nucleotide sequence of SEQ ID NO: 1[,]; or a DNA hybridizing with the DNA having a complementary nucleotide sequence of SEQ ID NO: 1 at 65C in the presence of 0.7 to 1.0M sodium chloride [under stringent conditions and coding for] and encoding a protein which confers an ability to grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine and 100 µg/ml lysozyme to a microorganism belonging to *Corynebacterium glutamicum*, wherein the hybridization further includes a step of washing under the condition of 65C by the use of solution containing 15 to 300 mM sodium chloride and 1.5 to 30 mM sodium citrate [has an activity of giving a lysozyme insensitivity to a lysozyme-sensitive microorganism belonging to *Corynebacterium glutamicum*].**
4. **(Amended) An isolated [A] DNA which is contained in a plasmid carried by FERM BP-6479 and codes for a protein which confers an ability to grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20**

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µg/ml thiamine and 100 µg/ml lysozyme to a microorganism belonging to *Corynebacterium glutamicum* [has an activity of giving a lysozyme insensitivity to a lysozyme sensitive microorganism belonging to *Corynebacterium glutamicum*].

20. (Amended) The DNA according to claim 1, wherein the microorganism is a mutant strain of *Corynebacterium glutamicum* which cannot grow in medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine and 50 µg/ml lysozyme [protein which has an activity of giving a lysozyme insensitivity to a lysozyme-sensitive microorganism belonging to *Corynebacterium glutamicum* is a protein having an activity of giving an insensitivity to 100 µg/ml lysozyme to a mutant belonging to *Corynebacterium glutamicum* and having a sensitivity to not more than 50 µg/ml lysozyme].

21. (Amended) The DNA according to claim 2, wherein the microorganism is a mutant strain of *Corynebacterium glutamicum* which cannot grow in a medium containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, 20 µg/ml thiamine and 50 µg/ml lysozyme [protein which has an activity of giving a lysozyme insensitivity to a lysozyme-sensitive microorganism belonging to *Corynebacterium glutamicum* is a protein having an activity of giving an insensitivity to 100 µg/ml lysozyme to a mutant belonging to *Corynebacterium glutamicum* and having a sensitivity to not more than 50 µg/ml lysozyme].

26. (Amended) The DNA according to claim 3, wherein the DNA is a DNA derived from a microorganism belonging to the genus *Corynebacterium* [*Cozynebacterium*].

30. (Amended) The DNA according to claim 3, wherein the DNA is a DNA derived from a microorganism belonging to *Corynebacterium glutamicum* [*Corynebacterium glutamicum*].

40. (Amended) A method for producing a protein, which comprises culturing the transformant of claim 36 in a medium, producing and accumulating the protein encoded by the DNA according to any one of claims 1, 20, 24 and 28 in the culture, and collecting the protein from the culture [wherein the protein is a protein which comprises the amino acid



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sequence of SEQ ID NO: 2, or a protein which comprises an amino acid sequence wherein one or more amino acids are deleted, substituted, or added in the amino acid sequence of SEQ ID NO: 2 and which has an activity of giving a lysozyme insensitivity to a lysozyme sensitive microorganism belonging to *Corynebacterium glutamicum*].

41. (Amended) A method for producing a protein, which comprises culturing the transformant of claim 37 in a medium, producing and accumulating the protein encoded by the DNA according to any one of claims 2, 21, 25 and 29 in the culture, and collecting the protein from the culture[, wherein the protein is a protein which comprises an amino acid sequence having 60% or more homology to the amino acid sequence of SEQ ID NO: 2 and which has an activity of giving a lysozyme insensitivity to a lysozyme sensitive microorganism belonging to *Corynebacterium glutamicum*].